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Cancer magister, Dana, the Dungeness crab was collected from the Columbia River estuary. The tissues from the crab were dissected and included the exoskeleton, endophragmal skeleton, mouth parts, stomach, gills, hepatopancreas, soft shell, muscles, and others. These tissues were analyzed for total and radioactive zinc, manganese and chromium using gamma ray spectroscopy and atomic absorbtion spectrometry.

Results indicated that ^{65}Zn was concentrated in the soft tissue, especially the muscle, probably because of metabolic utilization of zinc by these tissues. Manganese-54 was found to be concentrated mainly in calcified tissues presumably because of surface adsorbed $^{54}MnO_2$. It is also possible that manganese has substituted for calcium in the calcarious tissues. Surface adsorbtion as well as metabolic utilization of ^{51}Cr seem to be instrumental in its distribution in crab tissues.

The specific activity of ^{65}Zn was highest in the exoskeleton, hepatopancreas and stomach while the ^{54}Mn specific activity was highest in calcified tissues in all cases. Like the ^{51}Cr radioactivity, the ^{51}Cr specific activities were highest in the hepatopancreas and gills.

Levels of the radionuclides in the tissues changed throughout the year presumably as a result of changes in river flow, river productivity, physiological changes in the crab, and other factors. In general, levels of radioactivity and specific activities varied in much the same manner throughout the year.

The greatest specific activities were found in the inedible parts of the crab. The edible tissues, the muscles, had comparatively low specific activities. In no case would consumption of any tissue analyzed in this study constitute a health hazard according to the specifications set by the National Research Council (1962).

Distribution of ^{65}Zn, ^{54}Mn, and ^{51}Cr in the Tissues of the Dungeness Crab, Cancer magister Dana

by

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INTRODUCTION

Statement of the Problem

Radioactive wastes are added to the marine environment in many ways. The main sources of contamination are from (1) naturally occurring radioisotopes, (2) radionuclides originating from nuclear detonations, and (3) radioisotopes from nuclear power installations (Mauchline and Templeton, 1964). Naturally-occurring radioisotopes include those produced by the interaction of cosmic rays with the upper atmosphere and those which are isotopic components of the earth's crust. Nuclear detonations produce radioactive fallout particles . . . "when vaporized metals and fission products from the weapon and its associated structures mix with the surface material swept up into the cooling fireball" (Adams, Farlow and Schell, 1960). Nuclear reactors at Hanford convert ^{238}U to fissionable ^{239}Pu; in doing so Hanford becomes a source of radionuclides which are admitted to the North Pacific Ocean via the Columbia River.

The Columbia River is used to cool the Hanford reactors. As a result, certain trace elements in the river are exposed to a high neutron flux, capture neutrons, and become radioactive. Radioanalyses have shown that aquatic organisms living in the river

accumulate certain radionuclides. Zinc-65, ^{54}Mn, and ^{51}Cr as well as other radionuclides have been found in most biota living below Hanford.

Zinc and manganese are natural trace elements found in the river due to erosive and weathering processes; they are also abundant in the structure of the reactors. These elements, when subjected to neutron activation at Hanford become radioactive contaminants of the Columbia. Radioactive fallout is probably also a source of the ^{54}Mn (Folsom, Young, Johnson and Pillai, 1963). Sodium dichromate is added to the coolant waters at Hanford to inhibit corrosive processes; the chromium (VI) subsequently becomes radioactive as a result of neutron bombardment (Foster, 1961).

Thus ^{65}Zn, ^{54}Mn, and ^{51}Cr and other radionuclides produced at Hanford are transported downriver into the Pacific. Enroute they are concentrated and dispersed by biota, particulate matter, sediments, and water currents.

In recent years much attention has been given to the problem of organisms, especially edible ones, accumulating radionuclides from their environment. The National Research Council (1962) points out that although there is no evidence that this contamination has resulted in any adverse effects, it would be imprudent to suppose that harmful effects cannot eventually result from it. The following recommendation has been made concerning the disposal of

radioactive products. . .

> . . . that sea, water, organisms, and marine products are routinely collected both in and about the disposal areas and elsewhere and analyzed for radioactivity in such a way that the curie content and specific activity of the various radioisotopes can be determined (National Research Council, 1962).

Purpose of the Study

Marine, fresh water, and terrestrial products from below Hanford have been analyzed for radioactivity for many years. This has been done mainly out of concern for public health. While it is true that adverse effects may result from radiocontamination by any of these products, of primary concern should be those which are ingested by man (edible biota and water). It is prudent to keep in mind however, that the radioactive levels of aquatic foods are influenced by the activity of other aquatic substances such as sediments and particulate matter as well as water and other biota.

Many workers have studied organisms which accumulate radionuclides from the Columbia River and adjacent Pacific Ocean. Many of the animals investigated are of little or no importance to man in terms of food. This should be said with reservation since animals such as starfish, mussels, and barnacles may not be eaten by man to any extent but do serve as food for higher trophic level animals which are eaten by man. Even less work has been undertaken

to determine which parts or tissues of edible organisms accumulate the specific radionuclides.

The Dungeness crab, Cancer magister Dana is a commercially important seafood. Toombs (1966) analyzed this crab for ^{65}Zn, ^{95}Zr-^{95}Nb, and ^{103}Ru-^{106}Ru during the period January, 1962 through July, 1965. His analyses included all soft body parts of the crab lumped together. From his findings it is impossible to tell which soft tissues concentrated the radionuclides and therefore which tissues may represent a possible radiation hazard. This is important to know since only the muscle tissue from this crab is eaten by man.

The main purpose of this study is to examine the distribution of ^{65}Zn, ^{54}Mn, and ^{51}Cr (levels of radioactivity and specific activity) within the body of C. magister. The specific goals of the study are:

1. To determine which tissues are most likely to accumulate and concentrate the radionuclides of concern;

2. To gain a better understanding of factors which may influence the distribution of these radionuclides in the various tissues;

3. To describe seasonal variation of the radioactive levels in the crab tissues;

4. To determine which tissues, if any, may represent potential radiation hazards if eaten by man.

The Dungeness crab was chosen for this study because: (1) it is a commercially important seafood, (2) it is easy to capture, and

(3) it is relatively abundant in most seasons in the Columbia River estuary.

The Columbia River estuary was chosen for an area to study because: (1) it can be sampled economically, (2) facilities were made available to sample the area routinely and (3) previous studies in this area have provided background information pertinent to this project.

The elements chromium, zinc and manganese were selected for study because: (1) biota readily accumulate and concentrate some or all of these elements, (2) these three elements and their radioisotopes are prevalent in the estuary, (3) the radioisotopes have sufficiently long half-lives so that radioanalyses need not be rushed, and (4) equipment was available for both radioactive and total element analyses.

It is important that total as well as radioactive elemental concentrations are known since it is from the ratio of one to the other that the specific activity of a radionuclide may be determined. Specific activity is defined here as the ratio of the activity of radioactive isotope to concentration of total element. It can be written in the form of a simple equation, for example for ^{65}Zn:

$$\text{Specific activity } {}^{65}Zn = \frac{\text{pCi } {}^{65}Zn/\text{gm sample}}{\mu\text{ gm total Zn/gm sample}} = \frac{\mu\text{Ci } {}^{65}Zn}{\text{gm total Zn}}$$

The units in the equation are the same as those used in this thesis. The importance of the specific activity approach is emphasized in specifying maximum allowable concentrations of radionuclides in the sea:

> If the specific activity of the chemical elements in the sea in the environment of human food organisms are maintained below the allowable specific activities for those elements in the human body or human food, no person can obtain more than an allowable amount of radioactivity from the sea, regardless of his habits (National Research Council, 1962).

It should be made clear at this point that when considering the influence of the environment on the crab, that this environment includes sediments, water, and food. In recent years a food and water controversy has developed among radioecologists. Some workers contend water is the agent primarily responsible for the introduction of radionuclides into biota while others advocate that food is the primary source.

The element specific activity of any organism (or tissue) will depend on a number of factors including:

1. fluctuations in the specific activity of the tissue environment. If an organism remains in an environment of constant specific activity, then the specific activity of the organism will tend to approach but never exceed the specific activity of the environment.
2. the rate at which the element is utilized and turned over

by a tissue. Turnover time is defined as the time required for a tissue to completely utilize or turnover some quantity of some element. If in a tissue, the specific activity of an element is at steady state with an environment of constant specific activity, a tissue with a relatively long turnover time will have a lower specific activity than another tissue which has a shorter turnover time. The difference in the specific activities of the two tissues would be a function of radioactive decay.

3. trophic level of the organism. Generally, the higher the trophic level an organism occupies, the lower the specific activity of the organism. This is because the radionuclides decay enroute through the food chain and the radioactive proportion of the element will be smaller.

Other factors which may influence the element specific activity of tissues include metabolic rates, physiological condition, specificity of the element for the animal and others.

SURVEY OF THE LITERATURE

Biology and Ecology of *Cancer magister*

The Dungeness crab is named after a small fishing village on the straits of Juan de Fuca in Washington where the commercial fishing for this crab began. This crustacean belongs to the order Decapoda which includes the shrimp, crayfish, lobsters and crabs.

It is found from the Aleutian Archipeligo to Magdalena Bay, Mexico (Johnson and Snook, 1927). It has been reported that this crab lives from the intertidal zone to a depth of 93 fathoms (Phillips, 1935; Cleaver, 1949; Hipkins, 1947) usually upon a sandy bottom (Waldron, 1958) but has been reported to inhabit grassy (Hipkins, 1957), rocky (Cleaver, 1949), and muddy (Mackay, 1942) bottoms as well, the latter into which they have been observed burrowing.

The Dungeness crab is a scavanger and is known to exhibit cannibalism, especially upon other crabs in a soft-shelled stage (Cleaver, 1949; Mackay, 1942). Stomach examinations by MacKay (1942) revealed a variety of small crustaceans, bivalve mollusc remains, worms, and seaweed.

The temperature-salinity regime in which this crab lives, varies greatly. It has been reported to live in waters where the temperatures range from 3°C to 18°C and salinities from 11-22 ppt.

(Cleaver, 1949). October bottom temperature-salinity values for the Columbia River estuary at Chinook Point ranged from about 12°C and 32 ppt at high tide to about 14°C and 8 ppt at low tide (Kujala, 1966).

There is evidence to indicate that this crab follows the salt wedge into and out of the estuary. Waldron (1958), Cleaver (1949), and MacKay (1942) have shown that C. magister migrates into and out of estuaries and Cleaver (1949) has shown that this crab retreats before a freshet. This author has found that fewer crabs are caught on an ebb than on an incoming tide.

Ecdysis, the process of moulting, proceeds for this crab from May through November and young crabs moult more frequently than mature crabs (Pearson, 1908; MacKay, 1942; Cleaver, 1949). It is through the process of moulting that the crab and other crustaceans grow. Without moulting the rigid exoskeleton would prevent the expansion of body size. Ecdysis proceeds when the carapace splits open like a hinged box allowing the soft crab to crawl out backward.

The Dungeness crab is fished for commercially from Alaska to California at depths that range from 2 to 40 fathoms (Hipkins, 1957). During the 1964-1965 season, Oregon ports unloaded a total of 6,220,520 pounds (Pacific Marine Fisheries Commission Data Series, 1965) at an estimated value of $1,300,000 and for the

1965-1966 season 10,480,466 pounds were landed at an estimated value of $1,500,000 (Case, personal communication).

Zinc, Manganese, and Chromium in Marine Organisms

Distribution of Zinc, Manganese, and Chromium in Marine and Other Biota

Organisms in an aquatic environment tend to remove essential trace metals and elements from the environment and concentrate them in certain body tissues. If, as in the Columbia River estuary, a portion of these trace elements are radioactive, organisms will probably not discriminate between the radioactive and stable isotopes so long as the isotopes are in the same chemical form. Saltman (1958) demonstrated this with uptake of zinc isotopes by liver slices and Sather (1966a) demonstrated that crab tissue accumulated similar amounts of different chromium isotopes.

Zinc. Zinc in marine molluscs has been shown to be concentrated in the gills (Bodansky, 1920; Polikarpov, 1966; Chipman et al., 1958; Mori and Saiki, 1956). Bryan (1964) reports highest total zinc concentrations in the ovary of the lobster Homarus, followed by the hepatopancreas, excretory organs, gills, muscle, and blood. Marine fishes have been found to concentrate total zinc in the liver and spleen and in the male gonads during the breeding season (Polikarpov, 1966). Freshwater fishes were shown to

concentrate ^{65}Zn in the muscle tissue by Davis et al. (1958) and to be absent from the skeletal tissue of the sturgeon.

Vinogradov (1953) reports that Limulus, the horseshoe crab, concentrates zinc in the liver tissue. Bertrand and Vladesco (1923) report that the muscles of Platicarcinus pagurus contain more zinc than the gills and that the intestines contain the least.

Manganese. Polikarpov (1966) reports that related aquatic organisms concentrate similar amounts of manganese. Biota from waters adjacent to nuclear test sites have been examined for radioactive manganese. Phytoplankton contained little ^{54}Mn but trace amounts were found in zooplankton and in the bone, liver, and muscle of fish, particularly carnivores. The greatest concentrations were found in the liver of the giant clam Tridacna (Wangersky, 1961).

Many workers have found that manganese is associated with the hard parts of organisms more so than with the soft parts. John (1814) reported that the shell of the crayfish Potamobius fluviatilis contained measurable amounts of manganese (cited from Vinogradov, 1953). Polikarpov (1966) reports that manganese is concentrated in the shell of oysters to a much greater extent than in their soft parts. Muscle tissues concentrate comparatively little manganese in sea fishes according to Polikarpov (1966). Allen (1960) studied deposition rates of manganese on mollusc shells in the Clyde Sea and found that one millimeter of manganese was deposited every 25 to 60 years.

Geochemists are interested in the possibility of Mn^{++} replacing Ca^{++} in the calcite lattice of planktonic exoskeletons (Wangersky, 1961). This phenomenon may account for the distribution of manganese in the calcium carbonate sediments.

Studies with mammals in the laboratory have shown that manganese is essential for proper skin and bone development (Comar, 1948; Amdur et al., 1945).

Chromium. Relatively little work has been done concerning the distribution and biological importance of chromium, especially in aquatic biota.

Davis et al. (1958) found that ^{51}Cr was concentrated in Columbia River organisms of the lower trophic levels to a greater degree than in higher trophic levels. Relatively high ^{51}Cr concentrations were found by Davis et al. (1958) in the liver, spleen, and kidneys of the freshwater sucker Catostomus macrocheilus, and Watson et al. (1961) found ^{51}Cr in the soft parts of Mytilus sp. from near the mouth of the Columbia River. Sather (1966b), found that the chromium content of the carapace and hepatopancreas was significantly altered during various periods of the moulting cycle of Podophthalmus vigil and thinks it (Cr) a necessary metabolite. Polikarpov (1966) reports higher ^{51}Cr concentrations in the soft parts of the clam Mytilus galloprovincialis than in the shell.

Biology of Zinc, Manganese, and Chromium

The biochemical roles of zinc and manganese have been thoroughly studied especially in the higher vertebrates. Both elements are believed to be necessary for certain enzyme activities. The biological role of chromium appears more uncertain.

Zinc is associated with many enzymes including carbonic anhydrase (Keilen and Mann, 1944), alcohol dehydrogenase, glutamic and lactic dehydrogenases, carboxypeptidase, and alkaline phosphatase (Vallee, 1959). The enzymes with which manganese complex include dehydrogenases, decarboxylases, kinases, oxidases and peroxidases (Evans and Sorger, 1966). Chromium has been implicated in enzyme activation and oxidative and inhibitory metabolism (Sather, 1966b). Foster (1961) doubts that chromium is necessary for metabolic processes.

Hammen et al. (1962) determined enzyme activities in the mussel Modiolus demissus, the brachiopod Lingula reevi, and the oyster Crassostrea virginica. Enzymes present in all three included argenine deaminase, succinic dehydrogenase, carbonic anhydrase, arginase and urease. Whitely (1960) showed that a formate activating enzyme is present in the ovaries, testes, and digestive gland of Cancer magister and that manganese was effective in enzyme activation in this crab. Meenakshi and Sheer (1961) suggest that

C. magister contains a transglycosylase enzyme for glycogen synthesis and a hexokinase which catalyzes transphosphorylation. Beechey (1961) found what he believed to be nucleotides, cytochromes, and flavoproteins in the tissues of the crab Carcinus maenus. Flavoproteins are functional in the oxidation of succinate. Succinic dehydrogenase activity is reported by Ramamurthi (1966) in the gills and hepatopencreas of the freshwater crab Paratelphusa hydrodromus.

METHODS AND MATERIALS

Sampling Period

All of the crabs captured for this research were taken from the Columbia River estuary. Crabs were captured periodically throughout the year 1966-67. The first crabs were captured in March, 1966 and the last in February, 1967. Samplings were sporadic during the fall due to conflicts.

Sampling Gear

Collections were made from aboard the R/V Shoshone, a steel-hulled towboat modified for estuarine research. The twin engined craft cruises at about 14 knots, has an eleven foot beam and draws about 30 inches. It is outfitted with a small hydrographic winch, a larger hydraulic winch for benthic trawling, and a stern mounted A-frame.

Crabs were captured using a 22-foot shrimp type otter trawl and in crab pots (Fig. 1). By trawling for about twenty minutes, about six crabs would be caught. If more crabs were required, they were combined with crabs caught at the next sampling station or crab pots were used.

The crab pots are of the type commonly used by commercial

Figure 1. Crab pots used at Sand Island

fishermen. Each consists of a cylindrical steel framework about 36 inches in diameter and 14 inches deep. The entire framework is covered with stainless steel wire mesh. Funnel shaped entrances elevated from the bottom of the pot allow the crabs to enter with ease but make it very difficult to escape.

The pots were baited with clam necks or tuna innards, lowered to the bottom and marked with a buoy. Upon leaving the pots in the estuary overnight, capture of about a dozen crabs per pot could be expected.

Immediately after capture the crabs were placed in a pailful of the brackish estuarine water and frozen alive as soon as possible. Freezing was preferred to the use of formalin since the latter has been demonstrated by Tennant (1966) to leach trace amounts of zinc from fish tissue.

Sampling Sites

Crabs were collected from three locations in the estuary: Chinook Point, Sand Island and Buoy 10 (Fig. 2). If crab pots were used to capture the crabs, Sand Island was chosen as the collecting station since the water is only about 2 fathoms deep and the area is well protected from currents by pilings. In less protected locations strong channel currents would drag the buoys attached to the crab pots. The other two stations were usually sampled on the same day

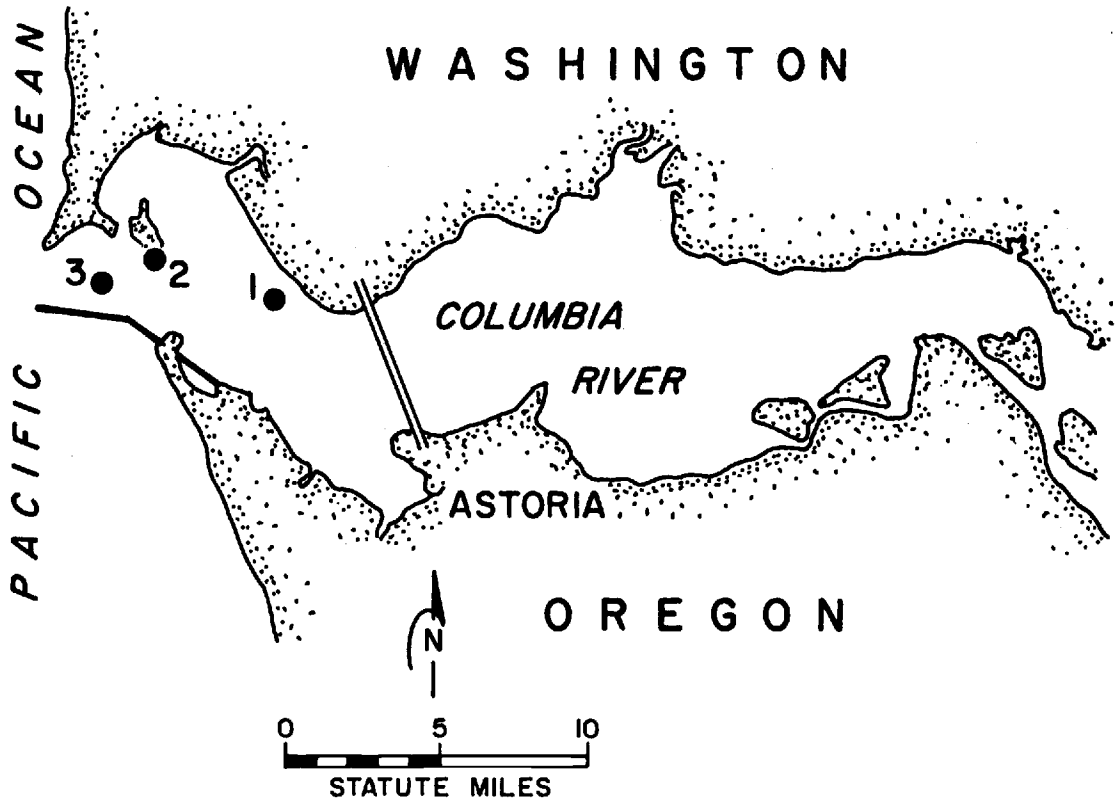

Figure 2. Sampling sites in the Columbia River estuary: (1) Chinook Point, (2) Sand Island, and (3) Buoy 10

using the otter trawl. If an insufficient number of crabs were taken from one station they were supplemented with those taken from the other station. No distinction was made between the crabs with respect to the station from which they were taken.

Sample Dissection

It was necessary to combine the tissues from two or more crabs to insure adequate sample size for radioanalysis and total element analysis. In all cases the tissues of crabs of similar size were combined. Thus, a particular gill sample would consist of all gills from two or three crabs of similar size which may have been caught at any one or all of the stations sampled.

The crabs were dissected into eight main tissue groups which are described in the following scheme. Anatomical terminology follows that of Pearson (1908).

Exoskeleton	Including carapace shell, branchiostegite shell, pereiopod shell and sternum shell
Mouth parts	Including antennae, mandibles, maxillae, and maxillipeds
Endophragmal skeleton	Consisting of thin calcareous plates forming an internal skeleton
Musculature	Including most flexors and extensors of the pereiopods

Alimentary canal	Consisting mainly of the cardiac fore-gut but included esophagus and mid-gut
Hepatopancreas	Including hepatopancreatic tissue from the antero-lateral, postero-lateral, and posterior lobes
Gills	Including the gills, flabella of the first maxillipeds, and the scaphognathites
Soft shell	Including only the soft shell

In addition to the above tissues, the entire abdomen, heart, gonads, setae from the carapace, carapace shell only, tergum shell only, leg shell only, dactylopodites, coelomic liquor, and whole crabs were analyzed.

Of all the different tissue fractions analyzed, only about half are discussed later in any length. Some of the dissections were exploratory to determine if differences in radioactivity could be observed. For instance the shell of the dactylopodites which are the terminal segments of the walking legs were analyzed separately from the rest of the shell. This was done because the dactylopodites are in direct contact with the substrate at all times while other portions of the shell may seldom be in contact with the substrate. The heart and gonadal portions were generally too small to give quantitative results. If the results of some of these analyses failed to produce pertinent information they were not included. Table 1 gives a breakdown of data pertinent to the dissections made.

Table 1. Tissue dissection scheme and sample data.

Group No.	Collection Data				Tissues Dissected and Analyzed																			
	Number of Crabs	Sex	Size (cm)	Date Collected	Exoskeleton	Leg Shell only	Carapace only w/setae	Branchiostegite w/o setae	Carapace only w/o setae	Setae	Mouth Parts	Gills	Stomach	Musculature	Hepatopancreas	Soft shell	Heart	Gonads	Endophragmal Skeleton	Dactylopodites	Abdomen	Coelomic liquor	Whole Analysis
I	2	?	?	3/26/66	X								X	X	X	X	X						
II	2	?	?	3/26/66	X						X	X	X	X	X	X	X	X	X				
III	2	?	15.5, 15.7	4/30/66	X						X	X	X	X	X	X	X		X				
IV	2	?	17.7, 17.4	4/30/66	X						X	X	X	X	X	X			X				
V	2	?	17.8, 18.4	4/30/66	X						X	X	X		X	X			X				
VI	2	?	16.3, 16.1	4/30/66	X						X	X	X	X	X	X	X		X				
VII	2	M	17.5, 16.3	4/30/66	X						X	X	X	X	X	X			X				
VIII	3	F	15.0 14.6, 12.3	6/ 7/66	X						X	X	X	X	X	X		X			X	X	
IX	2	M	13.8, 13.5	6/ 7/66	X						X	X	X	X	X						X	X	
X	3	M	11.9 13.9, 14.4	7/28/66	X						X	X		X	X					X	X		
XI	2	F	11.8, 11.8	7/28/66	X						X	X	X	X	X	X		X			X		
XII	2	F	12.4, 11.9	9/23/66		X	X				X	X	X										
XIII	2	M	16.5, 11.5	9/23/66		X	X				X	X		X									
XIV	4	M	14.8, 11.4 16.5, 16.5	9/23/66		X			X	X	X	X	X		X			X					
XV	2	M	13.2, 13.5	2/24/67	X			X		X	X	X	X	X	X								
XVI	5	/	7.5 approx.	2/24/67																			X

Concentration and Analysis

The tissues were placed in numbered porcelain crucibles. The samples were then dried at 70°C to constant weight in a drying oven, cooled and weighed. The samples (still in the crucibles) were then ashed for about twelve hours in a muffle furnace at about 450°C and ash weights were recorded after they had cooled in a dessicator. After ashing, the samples were divided so that they could be analyzed for both total and radioactive element concentration.

Radioanalysis

The ash fraction for radioanalysis was weighed on an analytical balance and put into a 15 cc plastic counting tube which was adjusted for counting geometry with sugar. The samples were analyzed for gamma activity in a 5 × 5 inch NaI (Tl) well crystal connected to a 512 channel analyzer (Nuclear Data, Series AT 130). The samples were counted for 100 minutes (400 minutes if the sample was of insufficient size or activity).

The analyzer sorts the radionuclides by assigning their energies to certain channels (0.01 mev/channel). An oscilloscope displays a spectrum of the sample, indicating which radionuclides are present and the approximate counting rate. An X-Y recorder plots this same spectrum on graph paper but on a larger scale with

decade lines which indicate the approximate counting values on a log scale. A digital readout is made, via an analog to digital converter, on an IBM typewriter with numbers representing the number of counts under the photopeaks present. A second form of digital readout is a punched tape on which is recorded the number of counts in each channel. (The punched tape and typeout provide exactly the same information; the typeout provides arabic numerals while the tape contains the numbers in binary code). Data stored on the punched tape are transferred to IBM punch cards and reduced by a CDC 3300 computer. Activity of the samples is determined by comparing the counting rate of the sample with the counting rate of standards of known activity. Background counts are subtracted from samples and standards prior to computation of activities. Thus the activities are correct for the time of collection. Activities can be computed long-hand without the aid of a computer. Such a computation is made in Appendix II.

Total Isotope Analysis

The portion of ash for total element analysis was weighed in 25 ml volumetric flasks. The flasks and ash were placed on a hot-plate in a ventilating hood. Approximately 8 ml of fuming nitric acid (90%) was added to each flask which was then boiled until near dryness, cooled, and diluted to volume with 0.36 N HCl.

The aqueous samples were aspirated via capillary tubing into the flame of an atomic absorbtion spectrophotometer (Perkin-Elmer model 303). Likewise, standard zinc, manganese and chromium solutions of known concentrations were aspirated.

Attenuation of a monochromatic light source is measured as it passes through the flame. The attenuation is due to a loss of energy, from the light, to ground state atoms in the flame; the ground state atoms being from the aqueous samples or standards. The amount of attenuation is directly proportional to the number of atoms in the flame. Total element concentrations were ascertained by comparing the amount of light attenuation by the unknowns to the amount by standards.

The attenuation was recorded as percent absorbtion on a 10 mv potentiometric strip chart recorder. Percent absorbtion values, weight of ash used, and concentration of standards were recorded on IBM punch cards. The data on the punch cards were then reduced by a CDC computer. The computer typeout provided sample concentrations as micrograms element per gram (ash) sample. Data reduction can be accomplished manually and is exemplified in Appendix II.

RESULTS

Samples of Dungeness crab, collected from the Columbia River estuary, were analyzed for radioactive and total zinc, manganese and chromium. The specific activities were computed for ^{65}Zn, ^{54}Mn and ^{51}Cr.

The X-Y readouts from the gamma ray spectrometer suggested that certain trends existed. For instance, the ^{51}Cr peak was noticeably present on the graphs of mouth parts, but was absent from the muscle graphs.

Such observations prompted this author to see if other trends existed. To gain a better picture of any trends, if they actually existed, the tissues were grouped. In doing so, it is hoped that a better understanding can be gained about factors which may influence the distribution of total and radioactive zinc, manganese and chromium in various crab tissues. Four factors are considered below and the tissues were grouped accordingly.

1. Some tissues are physically touching or in direct contact with the environment, while other tissues are not exposed to the environment. Thus the radioactivity of the tissue may be a function of the proximity of the tissues to the environment.
2. Many of the crab tissues are entirely calcified, others

are only slightly calcified while still others are entirely uncalcified. Thus the radionuclide content of the tissues may be a function of degree of tissue calcification.

3. Of the three elements being studied, zinc and manganese are known to be essential biological trace elements. Chromium is probably essential (Oser, 1965). It is likely that some of the crab tissues will utilize one or more of these elements more than other tissues. Thus, the radionuclide distribution may be a function of tissue metabolism.
4. In an annual cycle, the crab as well as its environment undergoes many changes.

For each of the four categories above, trends may exist for one or more of the radionuclides in question. The data obtained throughout the study has been averaged and plotted to detect these trends. The averages were weighed differently since some averages included more observations than others.

In all cases, the levels of radioactivity are expressed in pCi isotope/gm sample (ash) and the specific activities are reported in microcuries radioisotope/gm total isotope.

Proximity of the Tissue to the Environment

Three groups of tissues are considered here. One group, those tissues directly in contact with the environment, is composed

of the mouth parts, exoskeleton, and the gills. The setae (hair from underside of carapace) is plotted separately although it is an exposed tissue. The third group, those tissues not exposed to the environment, includes the muscles, hepatopancreas, endophragmal skeleton, and soft shell. The stomach has been omitted because it may be influenced by both the external and internal environment.

Radioactivity

Figure 3 shows that higher levels of ^{65}Zn are found in interior parts of the crab (those not exposed to the environment) than in those tissues exposed to the environment. There is more ^{65}Zn on or in the setae than in the other exposed tissues but less than in the interior tissues.

Levels of ^{54}Mn and ^{51}Cr show trends which are similar to one another. Perhaps the most striking thing in Figure 3 is that the levels of radioactivity in or on the setae are orders of magnitude higher than the levels of radioactivity in the other tissues for both ^{51}Cr and ^{54}Mn. Discounting the setae, the exposed tissue groups are indicated to concentrate ^{54}Mn and ^{51}Cr to a greater degree than do the interior tissues.

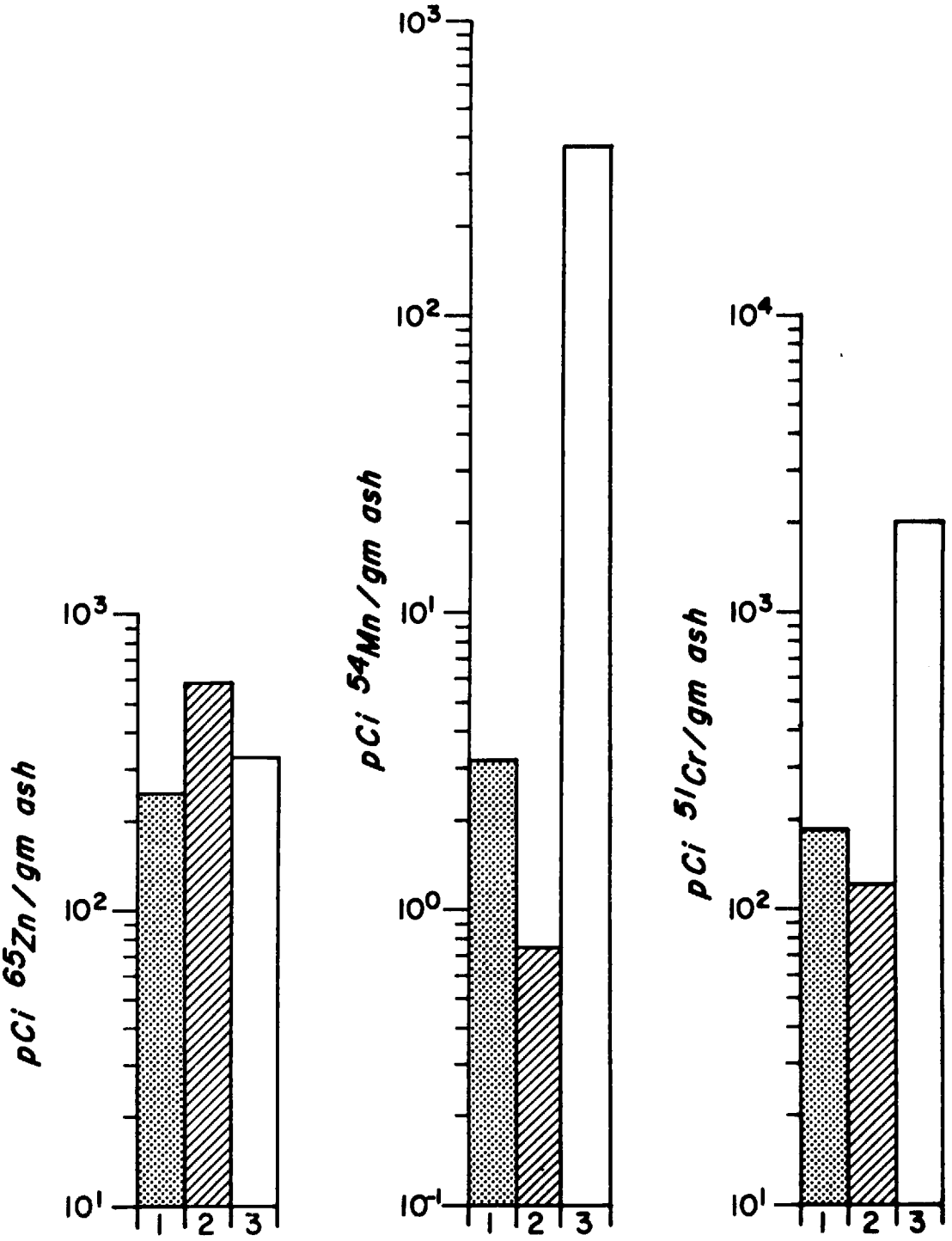

Figure 3. Levels of radioactivity in (1) tissues exposed to the environment, (2) tissues not exposed to the environment, and (3) the setae

Specific Activity

From Figure 4 it is seen that although the ^{65}Zn levels were lower in the exposed tissues the ^{65}Zn specific activity is higher there.

The specific activities of ^{51}Cr and ^{54}Mn show trends similar to the levels of radioactivity for the tissue. Chromium-51 specific activity could not be calculated for the setae due to insufficient sample size. It is apparent that the ^{51}Cr and ^{54}Mn specific activities are greater in the exposed tissues than in the interior tissues.

Degree of Tissue Calcification

The body tissues have been ranked from hard to soft i.e. calcified to non-calcified, in the following order: exoskeleton, mouth parts endophragmal skeleton, gills, stomach, soft shell, hepato-pancreas, and muscle. The gills and stomach are probably chitinous rather than calcareous (Pearson, 1908). The exoskeleton was freed of soft tissue during dissection, however, the mouth parts undoubtedly have bits of muscle and soft shell adhering to the sample and the endophragmal skeleton is undoubtedly contaminated by hepatopancreatic, muscular, and possibly gonadal tissue. The soft shell, was placed after the gills and stomach since it will eventually become calcified. The hepatopancreas and muscles are placed arbitrarily on the hardness scale since neither is subject to

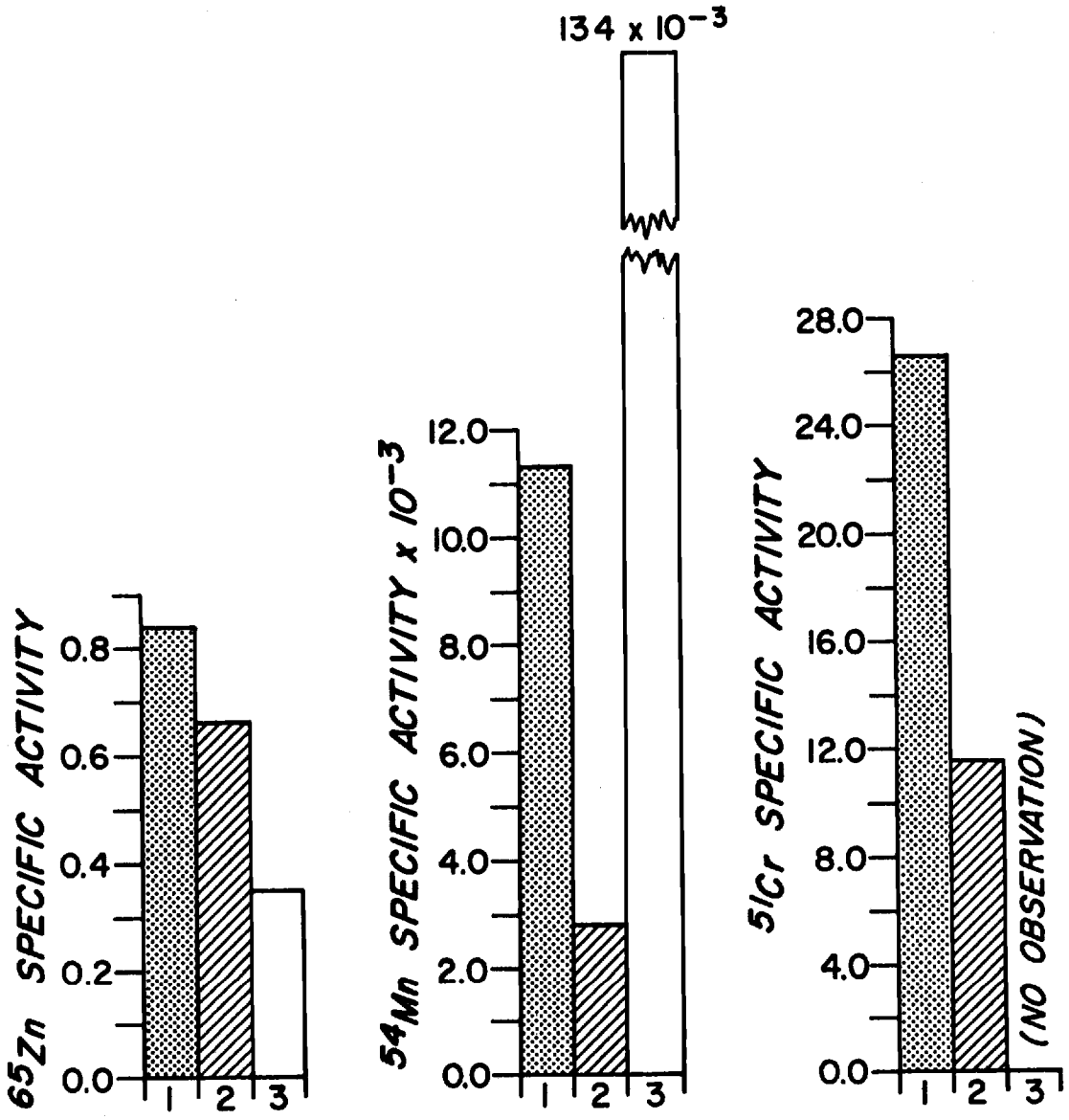

Figure 4. Specific activities in (1) tissues exposed to the environment, (2) tissues not exposed to the environment, and (3) the setae

calcification or chitinization.

Radioactivity

Figure 5 shows that the levels of ^{65}Zn are greater in the soft tissue rather than the calcified tissues. The levels of ^{54}Mn are strikingly associated with tissue calcification as Figure 5 shows. Chromium-51 concentrations do not seem to relate in any way to degree of tissue calcification.

Specific Activity

As Figure 5 shows, the highest ^{65}Zn specific activities are found in the exoskeleton, gills, and hepatopancreas. Low ^{65}Zn specific activities are indicated in the soft shell, mouth parts and endophragmal skeleton.

The specific activity of ^{54}Mn closely resembles the ^{54}Mn levels with the exception of the mouth parts and stomach.

The ^{51}Cr specific activities are similar to the ^{51}Cr levels in the tissues. The ^{51}Cr specific activity of the hepatopancreas was computed from only one observation.

It should be kept in mind that the placing of the tissue on the hardness scale presented here is arbitrary and therefore the trends shown are but generalizations.

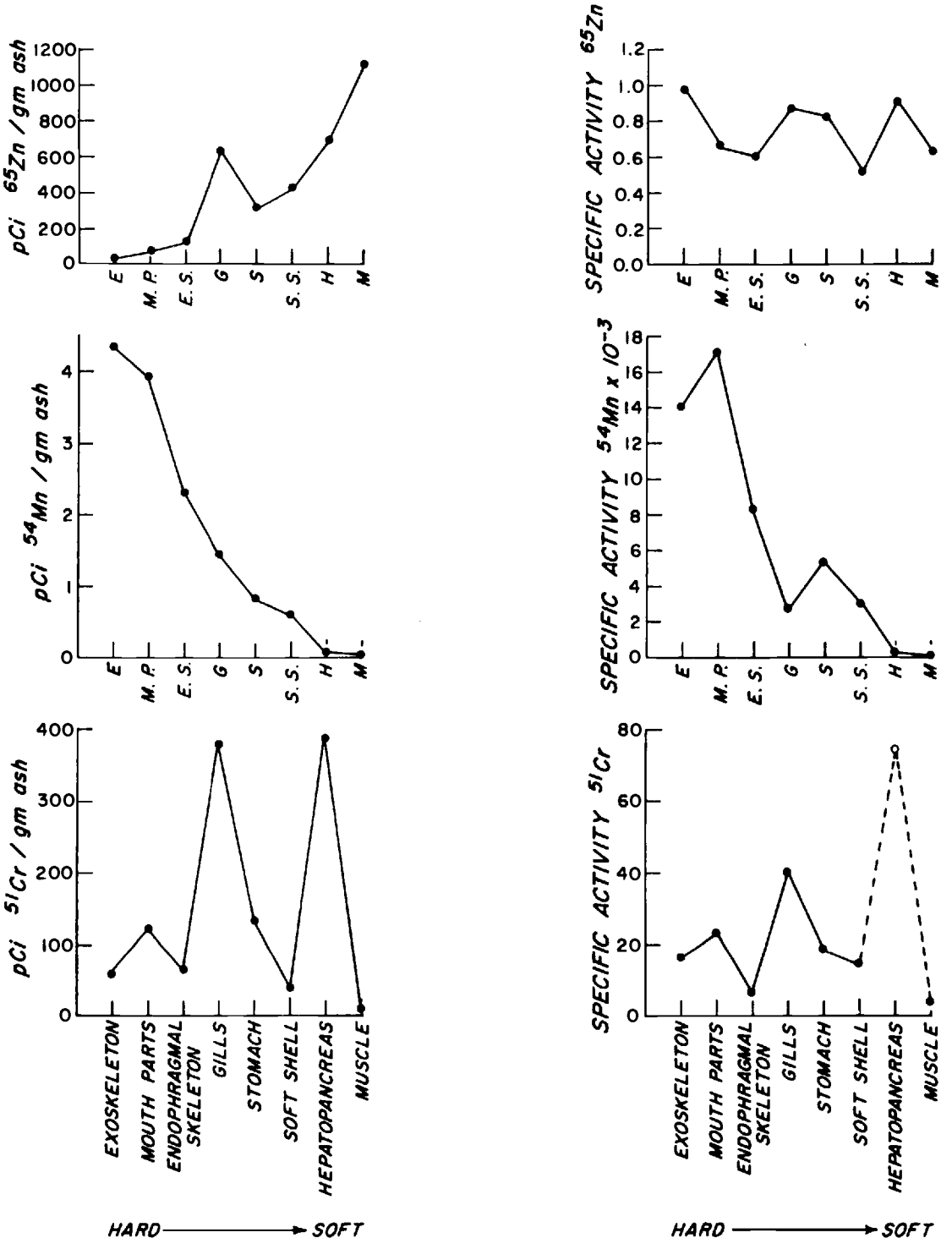

Figure 5. Variations in levels of radioactivity and specific activity as a function of tissue hardness

Tissue Metabolism of Zinc, Manganese, and Chromium

Crab tissues known (or highly suspect) to demonstrate high metabolic activity include: gills, hepatopancreas, muscles, soft shell, gonads and heart (Pearson, 1908; Tuttleand Schottelius, 1961; Florey, 1966). These have been grouped and their radioactive levels and specific activities averaged. Tissues which are considered to be relatively metabolically inert due to their low percentage of organic matter include the exoskeleton, mouth parts, and endophragmal skeleton. According to Nicol (1960) only 29% of the skeleton consists of organic matter, and about 80% of the inorganic portion is $CaCO_3$. Digestive as well as intracellular metabolism is carried on by the stomach, but since its radioisotope content is subject to influence from semi-digested products it is omitted.

Radioactivity

Figure 6 shows that ^{65}Zn is concentrated in the metabolically active tissues over eight times that concentrated in the non-metabolic tissues. Manganese-54 is concentrated in the metabolically inert tissues more than in the metabolically active tissues by a factor of almost three. Chromium-51 like ^{65}Zn is concentrated in the metabolically active tissues.

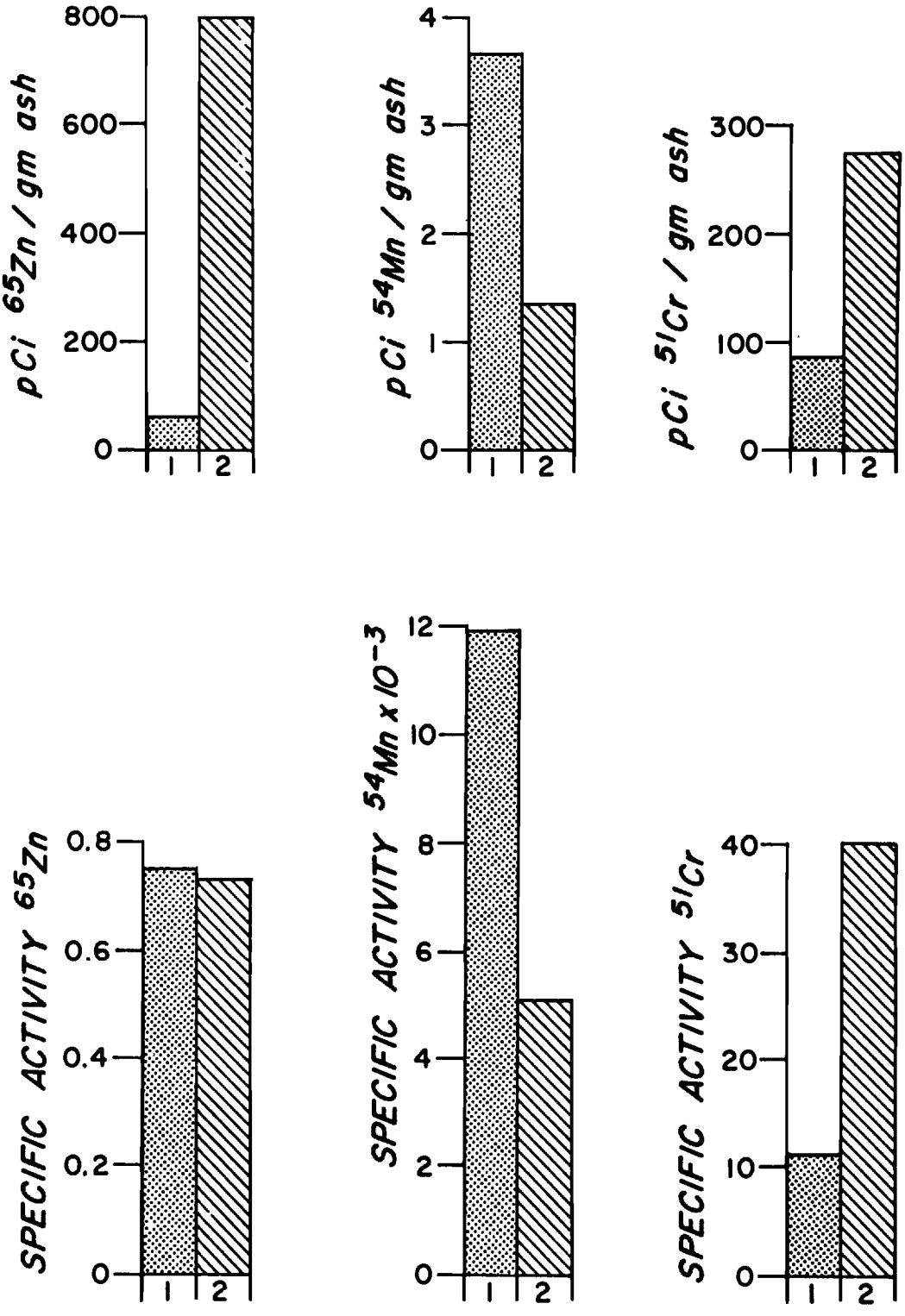

Figure 6. Levels of radioactivity (top) and specific activities (bottom) in (1) metabolically inert and (2) metabolically active tissues

Specific Activity

Figure 6 shows that the specific activity of ^{65}Zn is about the same for both metabolically active and inert tissues. The specific activity of ^{54}Mn on the other hand is more than twice as great for the metabolically inert tissues. The ^{51}Cr specific activity is four times greater in the rapidly metabolizing tissues.

Seasonal Variation

The levels of radioactivity and specific activities of ^{65}Zn, ^{54}Mn, and ^{51}Cr in crab tissues are plotted for 1966-67. The lines connecting the data points do not indicate radioactivity levels between the points but are meant to facilitate data interpretation. For some months certain tissues were not analyzed, consequently gaps appear in the curves. Some months single or few observations were taken making for poor statistical confidence.

From Figures 7, 8, and 9 four things should be noticed:

(1) The specific activities of the three radionuclides fluctuate throughout the year in the same manner as the radioactivity.

(2) In many cases peak specific activities and levels of radioactivity occur during high river discharge. (This is not to imply that the cause of the fluctuations in radioactivity in the crab is due to the same causes of specific activity fluctuations).

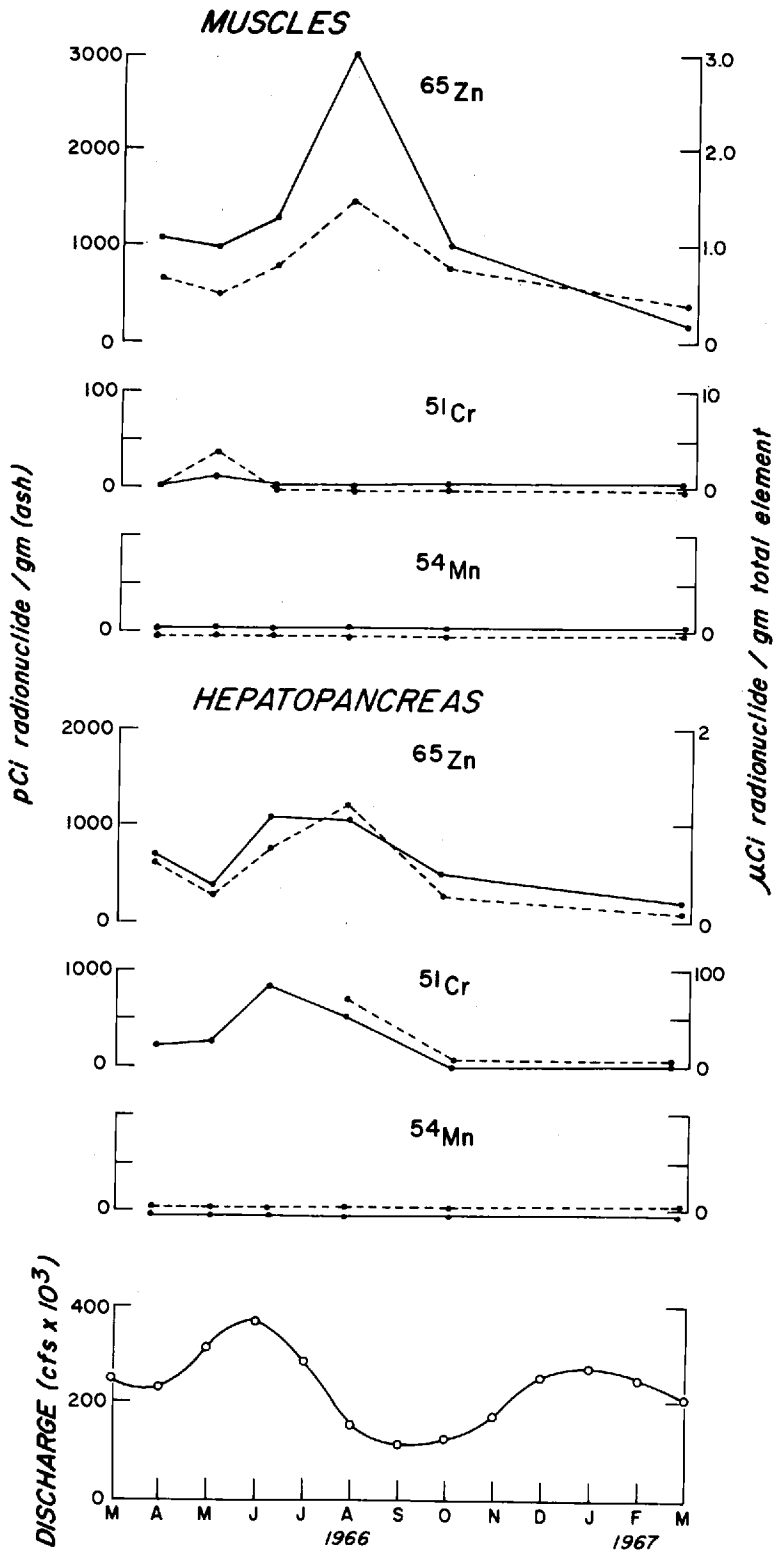

Figure 7. Fluctuations of specific activity (dashed lines) and radioactivity (solid lines) in soft crab tissues during the year 1966-1967

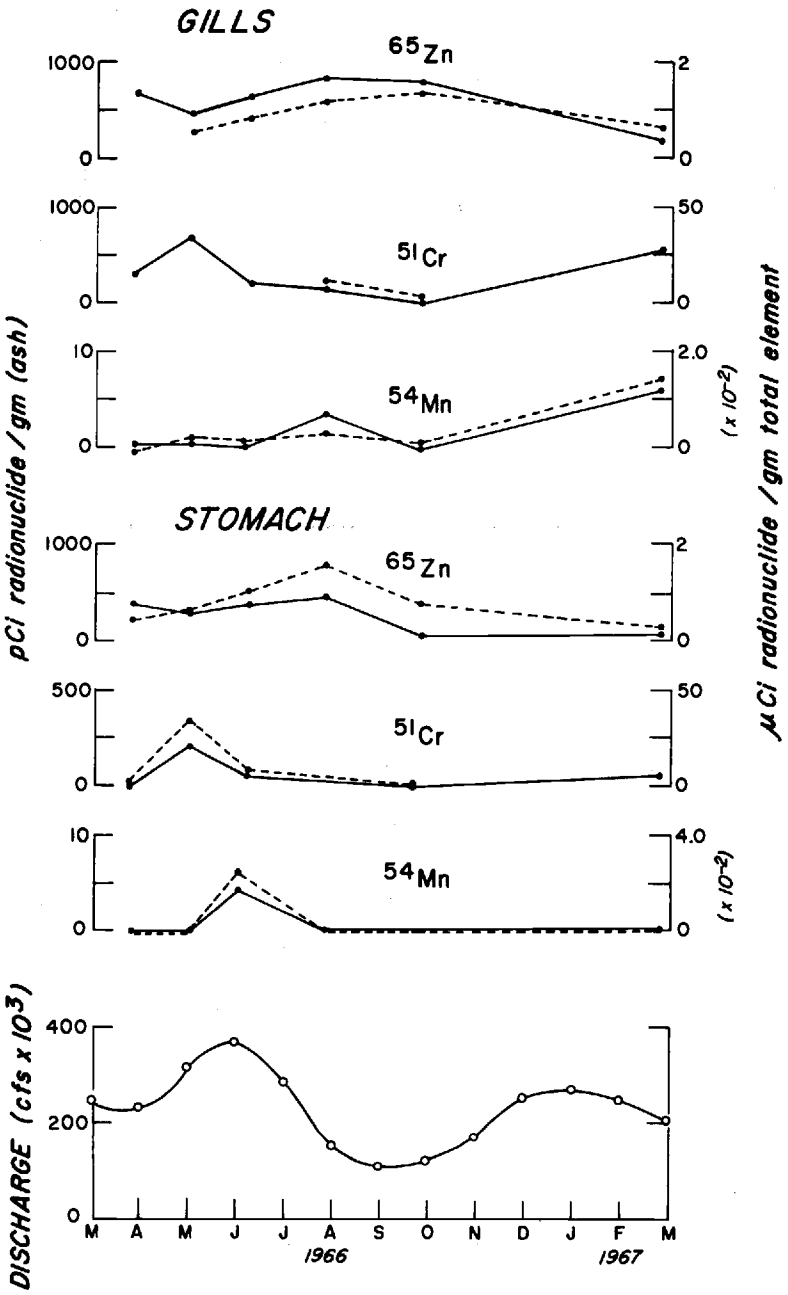

Figure 8. Fluctuations of specific activity (dashed lines) and radioactivity (solid lines) in soft crab tissues during the year 1966-1967

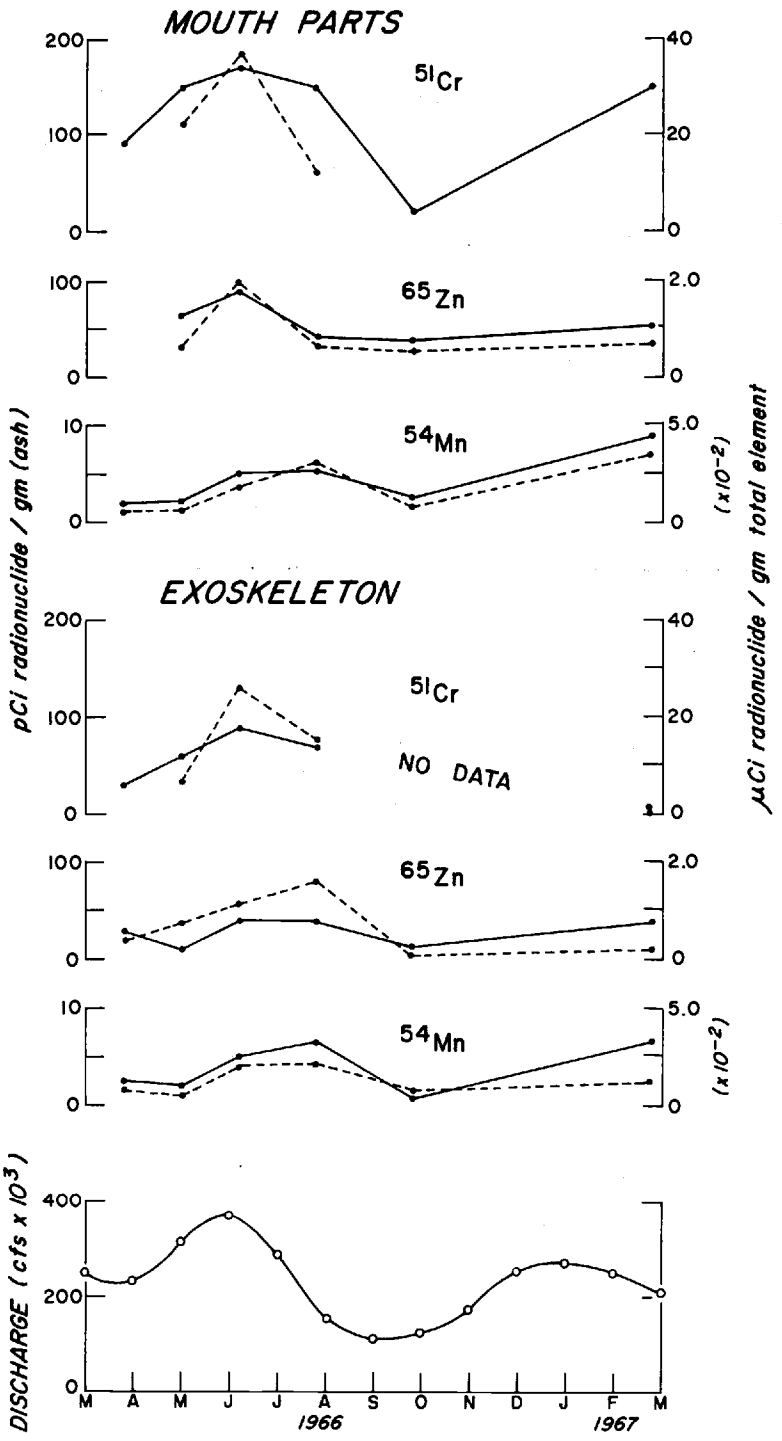

Figure 9. Fluctuations of specific activity (dashed lines) and radioactivity (solid lines) in hard crab tissues during the year 1966-1967

(3) Most of the radioactivity in the tissues is from ^{65}Zn and ^{51}Cr; ^{54}Mn contributed relatively little activity.

(4) Zinc-65 contributes the greatest amount of activity in the soft body parts (Figs. 7 and 8) while ^{51}Cr contributes the most in the calcareous body parts (Fig. 9).

DISCUSSION

Levels of Radioactivity

Zinc-65

It is seen from the preceeding graphs that the ^{65}Zn levels tend to:

1. be lower in tissues which are closest to or in contact with the environment and higher in interior tissues;
2. decrease as the degree of tissue calcification increases;
3. be greater in the metabolically active tissues rather than in the metabolically inert tissues;
4. be greater in all tissues during the summer months.

The results shown above suggest that levels of ^{65}Zn are associated negatively with closeness of tissue to the environment as well as with the degree of tissue calcification. The levels of ^{65}Zn did, however, relate positively to the degree of tissue metabolism.

That relatively little ^{65}Zn was found in either the exterior tissues or the calcified tissues may indicate that: 1) zinc is a relatively unessential constituent of these tissues compared to the soft tissues, 2) zinc is not subject to either adsorbtive or ion-exchange phenomena to any great degree, and 3) zinc probably does not substitute for other divalent cations which are abundant in the hard

exterior tissues. Conversely, manganese is thought to substitute for calcium in the calcite lattice (Wangersky, 1961), and chromium is associated with organisms having a high potential for adsorbtive uptake i. e. a high surface to volume relationship (Seymour and Lewis, 1964).

Zinc has been shown by Keilen and Mann (1944), Vallee (1962), and others to be a vital constituent of enzyme systems. The hard tissue parts are relatively metabolically inert during intermoult (Sather, 1966b) and it would be expected that zinc enzymes in the hard tissues would be reduced or absent. Surface adsorbtion of ^{65}Zn has been demonstrated by Osterberg et al. (1965) to play a minor role in the accumulation of this isotope.

Although there is relatively little information available on zinc metabolism in marine animals, there is evidence to suggest that metabolic utilization of zinc may be of primary importance in its distribution in the crab tissues. Evans (personal communication) believes that certain trace elements (including zinc) are utilized in different animals in a similar manner and that they are associated with similar enzyme systems.

Zinc makes up 0. 33% of the enzyme carbonic anhydrase (Keilen and Mann, 1944). Other zinc containing enzymes include dehydrogenases, phosphatases and peptidases (Vallee, 1959). Hammen et al. (1962) detected activity of the enzymes carbonic anhydrase, succinic

dehydrogenase and others in the mussel Modiolus demissus, the brachiopod Lingula reevi and the oyster Crassostrea virginica. Succinic oxidation was demonstrated in the hepatopancreas of the crab Carcinus maenus by Beechey (1961), and Ramamurthi (1966) reported succinic dehydrogenase in a lobster heart muscle.

Manganese-54

The ^{54}Mn detected in the crab was in many cases associated with different tissues than was ^{65}Zn. It was found that ^{54}Mn levels:

1. were higher in tissues exposed to the environment, i. e., ^{54}Mn levels tended to be a function of proximity of the tissues and the environment;
2. tended to increase as the degree of tissue calcification increased;
3. were lower in metabolically active tissues than in metabolically inert tissues.

That ^{54}Mn is associated with calcified tissues as well as tissues exposed to the environment is in fairly good agreement with some of the literature. Wangersky (1961) suggests that manganese will precipitate as MnO_2 in surface waters high in O_2 content and rich in particulate matter offering precipitation surfaces. Perhaps the exposed crab tissues serve as precipitation surfaces. Wangersky (1961) goes on to state that the precipitate of MnO_2, though

flocculent, adheres to every available surface, and that probably

> very little of the soluble Mn^{++} even in the oxidized form, would ever get to the open sea, since so much of the particulate matter within the estuary is of sufficient density to settle out within a relatively short distance (Wangersky, 1961).

It seems that MnO_2 on particulate matter which settles to the bottom may become associated with the exposed crab tissues. This may be the phenomena which Allen (1960) described. He found that manganese was deposited on the shells of living molluscs at a rate of one millimeter per 25 to 60 years. Allen (1960) concluded that the deposit was due to biological activity rather than by adsorbtion or by precipitation of particles.

Another method by which manganese may become associated with certain tissues is suggested by Wangersky (1961). He states that manganese is found in the calcareous skeletons of planktonic organisms and that manganese can substitute for calcium in the calcite lattice. Wangersky (1961) suggests that such a substitution and resolution at depth would account for the distribution of manganese in $CaCO_3$ sediments. Wangersky (1961) prudently points out though, that the manganese found associated with these calcareous skeletons may be a result of MnO_2 adsorbtion after deposition rather than crystal lattice substitution.

It would seem, in light of the literature reviewed, that the distribution of ^{54}Mn in the tissues is indeed a function of tissue

calcification as well as proximity of the tissue to the environment. It should not be overlooked that the endophragmal skeleton contained considerable amounts of ^{54}Mn even though this calcareous structure is not in contact with the crab environment. This may imply that manganese substitution for calcium is a mechanism of primary importance. Another implication here is that the manganese found, in the endophragmal skeleton as well as other calcareous tissues, may be routed into the tissues via food rather than directly from the water. It is also possible that manganese gains entry to the crab body via the gills and into the bloodstream and thence to the tissues. Mammalian studies have shown that manganese is absorbed through the gut wall when administered in inorganic form (Von Oettingen, 1935).

Measurable quantities of radioactive and stable manganese were also found in the soft tissues. Cotzias (1958) and Evans and Sorger (1966) have elucidated the role of manganese as a trace metabolite. As with zinc, manganese in some tissues is probably found as a metallo-enzyme complex.

Chromium-51

The levels of ^{51}Cr were seen to be highest in:

1. the tissues directly in contact with the water (especially the setae);

2. neither calcified nor non-calcified tissues (^{51}Cr levels do not appear to be related to tissue calcification);
3. the metabolically active tissues rather than the metabolically inert tissues.

Chromium from Hanford is introduced into the Columbia River as a hexavalent anion($Cr_2O_7^{2-}$) which probably dissociates in the river to yield CrO_4^{2-} (Cutshall et al., 1966). Any Cr (VI) that becomes reduced to Cr (III) is probably rapidly sorbed to particulate and subject to sedimentation (Cutshall et al., 1966). Thus any ^{51}Cr available to the crab for uptake is probably Cr (VI).

The highest ^{51}Cr concentrations were found in the setae, gills and hepatopancreas. Although there is some disagreement about the necessity of chromium as an essential trace metabolite, there is sufficient evidence to indicate that chromium is utilized for metabolic functions. It is unlikely though that the setae concentrate large amounts of chromium for metabolic utilization; rather, the ^{51}Cr found there is probably the result of adsorbtion. The hepatopancreas and the gills, on the other hand, probably do utilize chromium.

Oser (1965) states that trace amounts of trivalent chromium are necessary for proper glucose metabolism in rats and that chromium is poorly absorbed from foods; the largest part of ingested chromium may not be available for absorbtion. Oser (1965) classifies chromium as essential. Foster (1961) regards chromium as

unessential and rather that it is usually regarded as a toxic substance. Sather (1966b) implicated chromium to be a post-ecdysis agent which draws calcium to the required calcification sites in the crab Podophthalmus vigil. The muscle tissue analyzed in this study lacked measurable ^{51}Cr concentrations but the hepatopancreas and gills had high levels. Sather (1966b) suggests that the ^{51}Cr in the hepatopancreas is involved in glycogen replenishment.

Seymour and Lewis (1964) suggest that ^{51}Cr is mostly associated with organisms having a high surface to volume relationship. Thus, surface adsorbtion may be responsible for the high ^{51}Cr levels found in the setae which do indeed have a high surface area to volume relationship.

Seasonal Variation

Temporal fluctuations in the levels of radionuclides in different crab tissues may be caused by fluctuations in the amount of radionuclides available to the tissues and by the amounts utilized by the organism.

Perkins et al. (1966) found that high levels of radioactivity occurred in the Columbia River during high river discharge due to sediment scouring. Silker (1964) also found that the amounts of trace elements added to the Columbia from tributaries (upstream from reactors) was greatest during high river flow. It is therefore

likely that the peak radionuclide concentrations in the crab tissues result from the increased radionuclides available for uptake during the spring runoffs. The high specific activities which occur during this same time of the year are thought to be caused by a more rapid turnover of the radionuclides because of metabolic and other processes. Specific activities will be discussed more fully in a later section of this paper.

That ^{65}Zn and ^{51}Cr are the source of most of the radioactivity in the crab tissues is in agreement with other radiological surveys made on Columbia River waters. At Vancouver, Washington, 260 miles from the reactors, routine water samples have ^{51}Cr, ^{65}Zn, and ^{32}P accounting for 96.5 percent, 2.2 percent and 1.1 percent respectively of the total radioactivity in the water. In the estuary, 100 miles downstream from Vancouver, Seymour and Lewis (1964) found ^{51}Cr is 20 times more abundant than ^{65}Zn.

From Figures 7, 8, and 9 ^{51}Cr is more abundant than ^{65}Zn in only two tissues. This may seem odd in view of the relative isotope activities in the river which were reported in the last paragraph. The inconsistency can probably be explained in terms of concentration factors and metabolic utilization by the crab. Concentration factor refers to the concentration of an element within an organism (or tissue) divided by the concentration of the same element in the

environment. Table 2 is a portion of a table taken from Polikarpov (1966, p. 156).

Table 2. Concentration factors of stable Zn, Cr, and Mn in nature by groups of marine organisms.

Organism	Zinc	Chromium	Manganese
Brown algae	420-1,400	60	300-20,000
Sponges	30	800	3,000-95,000
Scyphomedusae	1,600	1,600	120
Malacostracan crustaceans	9,400-15,000	-	7,55
Molluscs (muscle)	2,600-40,000	-	-
Echinoderms	25-56	140-9,000	3,500-33,000
Echinoderm muscle	1,400	-	200
Fishes	280-15,500	2,000	95-126,000

Although the table does not give a complete picture, zinc is apparently concentrated to a much greater extent than is chromium. Manganese concentration factors are also relatively high even compared to zinc in some cases, and thus probably explains why ^{54}Mn is detectable in the concentrations reported.

Zinc-65 is more abundant in the soft tissues than ^{51}Cr while the reverse is true for the hard tissues. This is probably due to greater metabolic activity in the soft tissues as was discussed earlier in the paper.

Specific Activity

In choosing the Dungeness crab as an animal to study and the Columbia River estuary as a study location, a number of variables must be considered before discussing specific activity values.

The element specific activity of the crab undoubtedly results from the specific activity of its environment. The environmental specific activity is probably changing constantly in this case due to tidal cycles, land drainage, seasonal river flow rates, biological factors, and others. The crab may change its environment in another way. Studies mentioned previously have indicated that the crab migrates. Thus the crab may spend its time in waters of different specific activities for various durations by migrating in and out of the estuary.

A review of results necessary for a discussion of specific activities are given below.

The ^{65}Zn specific activities were:

1. higher in exposed than interior tissues;
2. didn't relate to degree of tissue calcification.

The ^{54}Mn specific activities were:

1. higher in exterior tissues than in interior tissues;
2. higher in calcified than uncalcified tissues.

The ^{51}Cr specific activities were:

1. higher in exposed than interior tissues;

2. not related to tissue calcification;

3. noticeably high in the setae, hepatopancreas, and gills.

In the following discussion a certain amount of grasping was necessary to bring in ideas which might be pertinent and valid in explaining the specific activity values obtained. Presented below are two hypothetical cases which, it is felt by this author, are likely to occur. These cases are "tailored" to a certain extent in an effort to shed meaningful light on the specific activities obtained.

Case I

Let us suppose that a crab were to migrate from an environment of low specific activity to one of high specific activity. Assume also that it was captured after it had resided in the new water parcel long enough to be influenced by the new specific activity but not long enough to equilibrate with it. Such a case may be expected if the ocean is of lower specific activity than the estuary, and if the crab were to make tidal migrations in and out of the estuary.

Foster (1959) has stated that animals of higher trophic levels would have lower specific activities than lower trophic levels in the same environment. This phenomenon is due to the radioactive decay of the radionuclides as they pass through the food chain. A similar effect may account for the specific activity variation within the crab

tissues. While some of the radionuclides in the interior tissues may enter the crab body via the gills, some will be ingested. Those ingested will probably be subject to food chain decay since the crab is a top trophic level feeder. Another consideration for ingested and gill absorbed radionuclides is that substantial radio-decay may occur in the time it takes to metabolically route the radionuclide from the environment to the tissue where it is utilized. Such radioactive decay would give rise to lower specific activities as the results suggest in some instances.

The exterior tissues on the other hand probably receive most of their radionuclides directly from the surrounding water. These tissues seemingly would be subject to a fairly rapid and constant replenishment of radionuclides from the water and therefore have higher specific activities as the results indicate.

Case II

Suppose that the same crab as above (Case I) had resided in this water parcel of relatively high specific activity long enough to equilibrate with the new environment prior to being caught. In such a case the above discussion could be altered.

The exterior tissues may still exhibit higher specific activities than the internal tissues for the same reasons stated above. A difference would probably be noticed, however, among the individual

internal tissues. A reason for this difference may be that trace element turn-over times will differ among tissues according to the individual tissue physiology. A tissue which is turning over a radionuclide faster than another tissue is will have a higher specific activity for the radionuclide than the second tissue. The low ^{54}Mn specific activity in the muscle tissue, for example, may be due to manganese being turned over slowly there. The relatively high ^{51}Cr specific activity in the gills on the other hand may result from chromium having a short turn-over time in the gill tissue.

Cancer magister as a Potential Food Hazard

The only portion of the Dungeness crab which must be considered as a source of hazardous radiocontamination is the muscle tissue. Neither ^{51}Cr nor ^{54}Mn appear to be concentrated in muscle tissue either. Therefore, it would appear that of the three radionuclides discussed, ^{65}Zn would be the likeliest source of damaging radiation if crab meat were eaten in excess. However, when the concept of specific activity is considered as recommended by the National Research Council (1962), ^{65}Zn becomes relatively unimportant as a contaminant. The highest ^{65}Zn specific activities were found in the exoskeleton during the summer months.

The National Research Council (1962) has stated that the maximum permissible ^{65}Zn specific activity for the human body is

26 μCi ^{65}Zn/gm total Zn. The highest ^{65}Zn specific activity found in crab muscle tissue is at least an order of magnitude lower than this. The maximum permissible ^{51}Cr specific activity for the human body is almost 4,000 times higher than the ^{51}Cr specific activity found in the crab muscle and the ^{54}Mn specific activity of crab muscle is at least two orders of magnitude lower than the permissible ^{54}Mn specific activity. These findings indicate that there is no present danger from radiocontamination of crabmeat in the Columbia River estuary.

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APPENDICES

APPENDIX I

The following data is presented for each tissue sample analyzed. Specific activity values were computed from the appropriate values below. The tissue groups discussed in the thesis also derive their plotted averages from these figures.

Radioactive and total element concentrations in samples analyzed.

Sample No.	Data Collected	Tissue	μgm Zn/gm (ash)	pCi ^{65}Zn/gm (ash)	μ gm Mn/gm (ash)	pCi ^{54}Mn/gm (ash)	μ gm Cr/gm (ash)	pCi ^{51}Cr/gm (ash)
1	3-26-66	Exoskeleton	62.5	52.0	376	5.07	-	40.2
2	"	Endophragmal Sk.	514	375.0	282	-	19.1	-
3	"	Gills	-	-	-	-	-	-
4	"	Heart	-	-	-	-	-	-
5	"	Gonad	-	-	-	-	-	-
6	"	Heart	-	-	-	-	-	-
7	"	Muscle	1452	1123	70.0	0	7.73	0
8	"	Gills	-	713	34.0	0	-	315
9	"	Soft shell	709	619	224	2.75	18.3	0
10	"	Endophragmal Sk.	157	56.0	121	1.94	-	21.8
11	"	Stomach	1080	395	94.0	0	-	0
12	"	Muscle	1456	1187	86.0	0	4.30	0
13	"	Hepatopancreas	918	698	144	1.15	-	235
14	"	Soft Shell	1218	635	79.0	0	2.0	0
15	"	Mouth Parts	95.0	22.4	257	1.91	-	95.7
16	"	Hepatopancreas	1107	727	97.0	0	-	231
17	"	Muscle	2101	1072	68.0	0.324	2.04	65.0
18	"	Exoskeleton	35.0	11.7	302	2.47	-	21.1
19	"	Muscle	2030	887	35.0	0	-	0
20	"	Stomach	610	393	119	0	14.2	0
21	4-20-66	Exoskeleton	48.0	-	511	-	5.59	-
22	"	Mouth Parts	123	92.9	422	0	8.98	185
23	"	Endophragmal Sk.	130	139	157	5.77	13.6	86.4
24	"	Gills	770	397	132	0	-	702
25	"	Stomach	309	373	76.0	0	11.8	228
26	"	Muscle	1853	1578	358	0	3.17	0
27	"	Hepatopancreas	1107		72.0	-	-	-
28	"	Soft Shell	715	82.9	-	0	-	46.4
29	"	Gonad	-	-	81.0	-	-	-
30	"	Muscle	899	615	31.0	0	5.35	0

Sample No.	Data Collected	Tissue	μ gm Zn/gm (ash)	pCi ^{65}Zn/gm (ash)	μ gm Mn/gm (ash)	pCi ^{54}Mn/gm (ash)	μ gm Cr/gm (ash)	pCi ^{51}Cr/gm (ash)
31	4-20-66	Muscle	2006	1227	221	0	5.65	0
32	"	Exoskeleton	17.0	22.3	76.0	1.97	-	83.0
33	"	Hepatopancreas	505	389	81.0	0	-	253
34	"	Stomach	578	509	268	0	14.6	589
35	"	Mouth Parts	131	92.2	77.0	1.70	7.97	147
36	"	Gills	790	575	203	0	-	299
37	"	Endophragmal Sk.	104	70.1	37.0	2.01	5.07	40.3
38	"	Muscle	2125	1286	38	0	5.08	0
39	"	Soft Shell	808	514	24.0	0	10.1	0
40	"	Muscle	2054	747	463	0.367	14.3	0
41	"	Exoskeleton	13.0	11.2	163	2.73	7.70	59.3
42	"	Mouth Parts	39.0	21.7	129	3.40	6.86	142
43	"	Gills	692	442	109	0	-	162
44	"	Stomach	571	321	33.0	0	4.38	149
45	"	Muscle	2172	782	76.0	0	2.13	0
46	"	Hepatopancreas	834	612	44.0	0	-	248
47	"	Soft Shell	763	365	477	0	3.38	73
48	"	Exoskeleton	30.0	9.28	67.0	6.03	5.83	42.2
49	"	Hepatopancreas	629	361	51.0	0	-	127
50	"	Soft Shell	743	341	44.0	0	2.46	66.7
51	"	Muscle	2105	796	515	0	2.15	0
52	"	Gills	500	299	121	4.37	-	1875
53	"	Stomach	550	207	50.0	0	10.5	42.0
54	"	Heart	798	676	445	0	18.5	0
55	"	Endophragmal Sk.	182	59.0	233	4.04	16.2	62.7
56	"	Mouth Parts	69.3	50.2	67.0	2.92	10.3	145
57	"	Muscle	2287	716	67.0	0	-	0
58	"	Hepatopancreas	504	509	-	-	-	-
59	"	Soft Shell	710	-	44.0	-	4.20	-
60	"	Mouth Parts	-	63.4	207	1.47	4.20	143
61	"	Stomach	233	196	41.0	0	4.80	82.4

Sample No.	Data Collected	Tissue	μ gm Zn/gm (ash)	pCi ^{65}Zn/gm (ash)	μgm Mn/gm (ash)	pCi ^{54}Mn/gm (ash)	μ gm Cr/gm (ash)	pCi ^{51}Cr/gm (ash)
62	4-20-66	Gills	681	534	78.0	0	-	439
63	"	Muscle	2164	907	32.0	0	7.48	76.6
64	"	Muscle	2048	843	19.0	0	5.21	0
65	"	Exoskeleton	14.3	14.9	247	2.10	5.23	51.0
66	"	Ednosphragmal Sk.	205	92.1	67.0	0	5.72	39.0
67	"	Heart	-	-	-	-	-	-
68	6-7-66	Abdomen	227	190.3	353	8.02	4.00	339
69	"	Exoskeleton	40.7	34.5	164	3.60	11.7	87.5
70	"	Muscle	2377	11.72	48.0	0	5.26	0
71	"	Hepatopancreas	-	-	-	-	-	-
72	"	Gills	665	539	120	0	-	363
73	"	Mouth Parts	99.2	83.9	162	3.39	7.91	183
74	"	Stomach	173	94.8	132	0.93	10.0	124
75	"	Heptopancreas	723	636	59.0	0	-	417
76	"	Soft Shell	1076	759	44.0	0	2.51	90.1
77	"	Ovary	1659	1365	99.0	0	-	137
78	"	Coelomic Fluid	100	112	89.4	2.38	7.12	91.3
79	"	Exoskeleton	36.7	60.4	304	6.90	1.93	90.9
80	"	Muscle	1406	1544	51.0	0	-	0
81	"	Soft Shell	1153	-	109	-	7.49	-
82	"	Hepatopancreas	1161	1571	80.0	0	-	1328
83	"	Stomach	412	729	168	8.32	10.1	0
84	"	Gills	659	788	181	0	-	0
85	"	Abdomen	136	156	456	6.37	8.82	2.29
86	"	Mouth Parts	86.8	104	346	6.37	3.05	1.55
87	"	Coelmic Fluid	326	401	50.0	0	2.19	283
88	7-28-66	Exoskeleton	27.0	40.5	212	5.15	3.45	76.8
89	"	Dactylopodite	52.3	-	185	-	7.32	-
90	"	Mouth Parts	84.0	30.6	180	8.30	12.1	140
91	"	Hepatopancreas	560	671	44.0	0	-	177
92	"	Gills	470	606	93.0	6.80	8.69	81.0

Sample No.	Data Collected	Tissue	μ gm Zn/gm (ash)	pCi ^{65}Zn/gm (ash)	μ gm Mn/gm (ash)	pCi ^{54}Mn/gm (ash)	μ gm Cr/gm (ash)	pCi ^{51}Cr/gm (ash)
93	7-28-66	Abdomen	-	181	161	0	-	206
94	"	Muscle	947	-	24.0	-	-	-
95	"	Hepatopancreas	352	349	20.0	0	-	108
96	"	Exoskeleton	25.0	49.0	359	8.24	7.53	69.1
97	"	Hepatopancreas	897	2315	67.0	0	19.0	1414
98	"	Soft Shell	710	-	43.0	-	6.95	-
99	"	Stomach	248	478	152	0	18.5	453
100	"	Gills	-	-	-	-	10.39	-
101	"	Mouth Parts	-	171	303	5.87	7.09	149
102	"	Muscle	1991	3061	57.0	0	-	0
103	"	Ovary	956	-	86.0	-	-	-
104	"	Abdomen	273	424	572	14.3	17.6	290
105	"	Gills	924	1085	170	0	-	252
106	9-23-66	Exoskeleton (lost)	-	-	-	-	-	-
107	"	Muscle (lost)	-	-	-	-	-	-
108	"	Hepatopancreas (lost)	-	-	-	-	-	-
109	"	Stomach	93.01	73.6	99.0	0	-	0
110	"	Soft Shell (lost)	-	-	-	-	-	-
111	"	Gills	-	169	-	0	-	0
112	"	Exosk. w/o Setae	99.0	1.32	104	0.234	-	0
113	"	Mouth Parts	111	34.5	190	0.776	-	8.0
114	"	Ovary (lost)	-	-	-	-	-	-
115	"	Leg Shell	99.0	12.2	143	1.01	-	0
116	"	Muscle	1322	1120	31	0	-	0
117	"	Carapace (lost)	-	-	-	-	-	-
118	"	Gills	1056	1467	54.0	0	-	0
119	"	Mouth Parts	186	109	1.93	2.41	-	8.57
120	"	Leg Shell (lost)	112	-	225	-	-	-
121	"	Muscle (lost)	-	-	-	-	-	-
122	"	Hepatopancreas	813	508	80	0	-	0
123	"	Stomach (lost)	-	-	-	-	-	-

Sample No.	Data Collected	Tissue	μ gm Zn/gm (ash)	pCi ^{65}Zn/gm (ash)	μ gm Mn/gm (ash)	pCi ^{54}Mn/gm (ash)	μ gm Cr/gm (ash)	pCi ^{51}Cr/gm (ash)
124	9-23-66	Soft Shell (lost)	-	-	-	-	-	-
125	"	Mouth Parts (lost)	-	-	-	-	-	-
126	"	Gills (lost)	-	-	-	-	-	-
127	"	Carapace (lost)	-	-	-	-	-	-
128	"	Setae (lost)	-	-	-	-	-	-
129	2-24-67	Exoskeleton	195	39.1	478	6.60	-	0
130	"	Muscle	993	439	143	0	-	0
131	"	Hepatopancreas	506	295	119	0	-	0
132	"	Stomach	385	83.3	136	0	-	41.4
133	"	Soft Shell	604	236	196	4.75	-	0
134	"	Mouth Parts	158	54.5	252	9.15	-	1.50
135	"	Gills	492	236	397	6.00	-	522
136	"	Setae	924	327	2782	373	-	2021
137	"	Carapace	123	24.8	293	4.33	-	0
138	"	Coelomic Fluid	769	140	183	0	-	0
139	"	Whole Crab	327	95.9	105	1.31	-	0
140	"	Gonad	854	191	-	8.4	256	253

APPENDIX II

To illustrate how gamma activities are computed "longhand" after they have been counted, a hypothetical sample computation will be made. The method is from Covell (1959). Figure 10 shows the gamma spectrum of a tissue sample. This computation will be made to determine the ^{54}Mn activity. The ^{54}Mn photopeak is enlarged by graphing it manually on linear graph paper (Fig. 11).

1. Select an equal number of channels, n, on each side of the center channel of the photopeak, and coming about half way down.
2. Sum up the total number of counts between and including the selected channels (a+b).
3. Subtract from the total number of counts, the counts in the parallelogram (shaded area).
4. Compare the remaining counts (those in the unshaded area) to those in a known standard, determined in a similar manner.
5. From the known activity of the standard compute the activity of the unknown.

$$\text{Unshaded area} = \sum_{a_n}^{b_n} C - (2n+1)/2 \times (a_n + b_n)$$

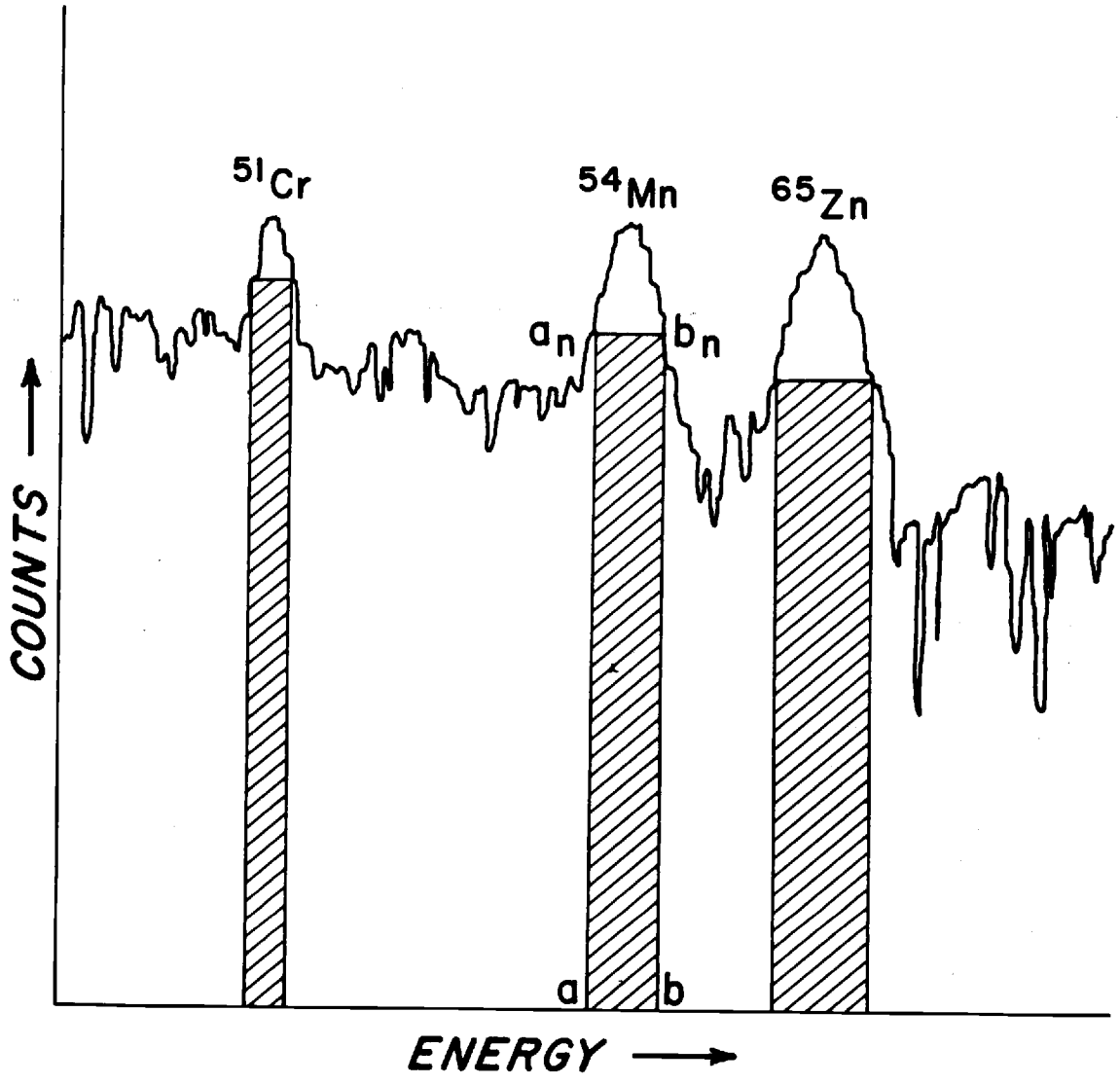

Figure 10. Gamma spectra of ^{51}Cr, ^{54}Mn, and ^{65}Zn from crab tissue with channel limits (a) and (b); (a_n) and (b_n) indicate counts at these limits

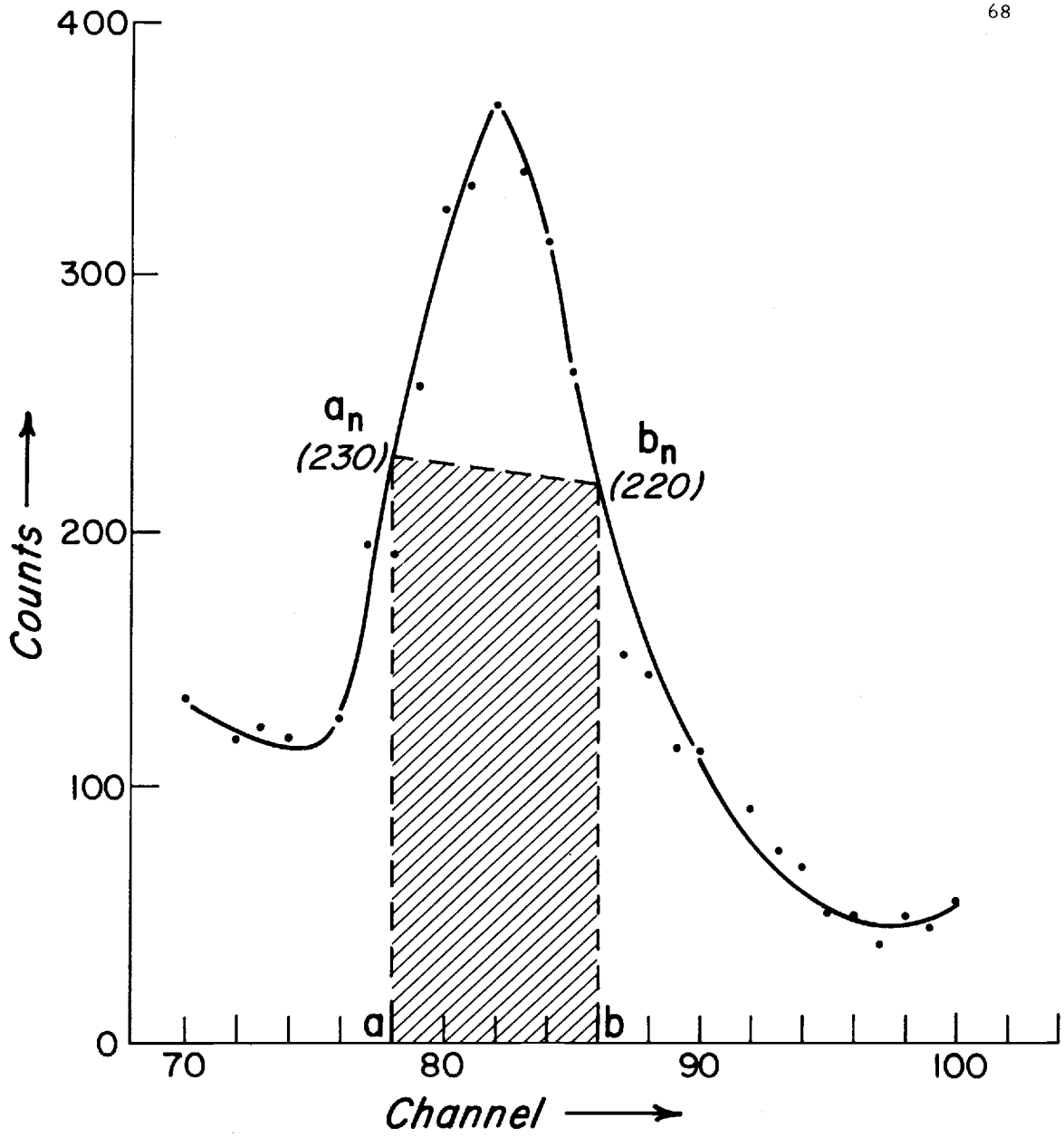

Figure 11. Graphed ^{54}Mn photopeak showing channel limits (a) and (b), and number of counts at these limits i. e., (a_n) and (b_n)

where: ΣC = Total number of counts

n = number of selected channels on either side of the center channel of the photopeak

a_n, b_n = number of counts in channel a, or b.

The following information is provided to make the computation:

1. The radionuclide of concern is ^{54}Mn;
2. The activity of the standard is 5.71×10^2 at the time of counting;
3. The number of counts at (a) in the standard photopeak is 100 and at (b) is 75;
4. The total number of counts $\sum_{a_n}^{b_n} C$ in the standard is 1,082;
5. The weight of the sample (ash) is 2.3 gm.

The following information is ascertained from Figure 11:

1. The number of counts in the unknown photopeak at (a) is 230 and at (b) is 220;
2. The total number of counts $\sum_{a_n}^{b_n} C$ in the unknown is 2,649.

The remaining required computation would be:

A. Standard counts in unshaded area

1. $n = 4$; $(2n + 1)/2 = 4.5$
2. $(a_n + b_n) = 175$ counts

3. $4.5 \times 175 = 783$ counts

4. $1,082 - 783 =$ 299 counts

B. Unknown counts in unshaded area

1. $n = 4$; $(2n + 1) = 4.5$

2. $(a_n + b_n) = 225$ counts

3. $4.5 \times 225 = 2,025$ counts

4. $2,649 - 2,025 =$ 624 counts

C. Unknown ^{54}Mn activity

1. Standard-unknown ratio = $624/299 = 2.06$

2. Standard activity = 5.71×10^2 pCi

3. Weight of unknown = 2.3 gm (ash)

4. $2.06 \times 5.71 \times 10^2$ pCi = 11.76 pCi

5. 11.76 pCi $^{54}Mn/2.3$ gm = 5.1 pCi ^{54}Mn/gm ash

Data reduction for total element determinations can also be accomplished without the aid of a computer. Such a determination is here illustrated for total zinc in crab tissue.

A standard curve is made by plotting absorbance vs concentration (percent absorbtion is converted to absorbance to correct for the non-linearity of the deviation from Beer's law). Figure 12 shows the standard curve and the determined unknown (sample concentration). To complete the computation, sample weight and volume must be considered:

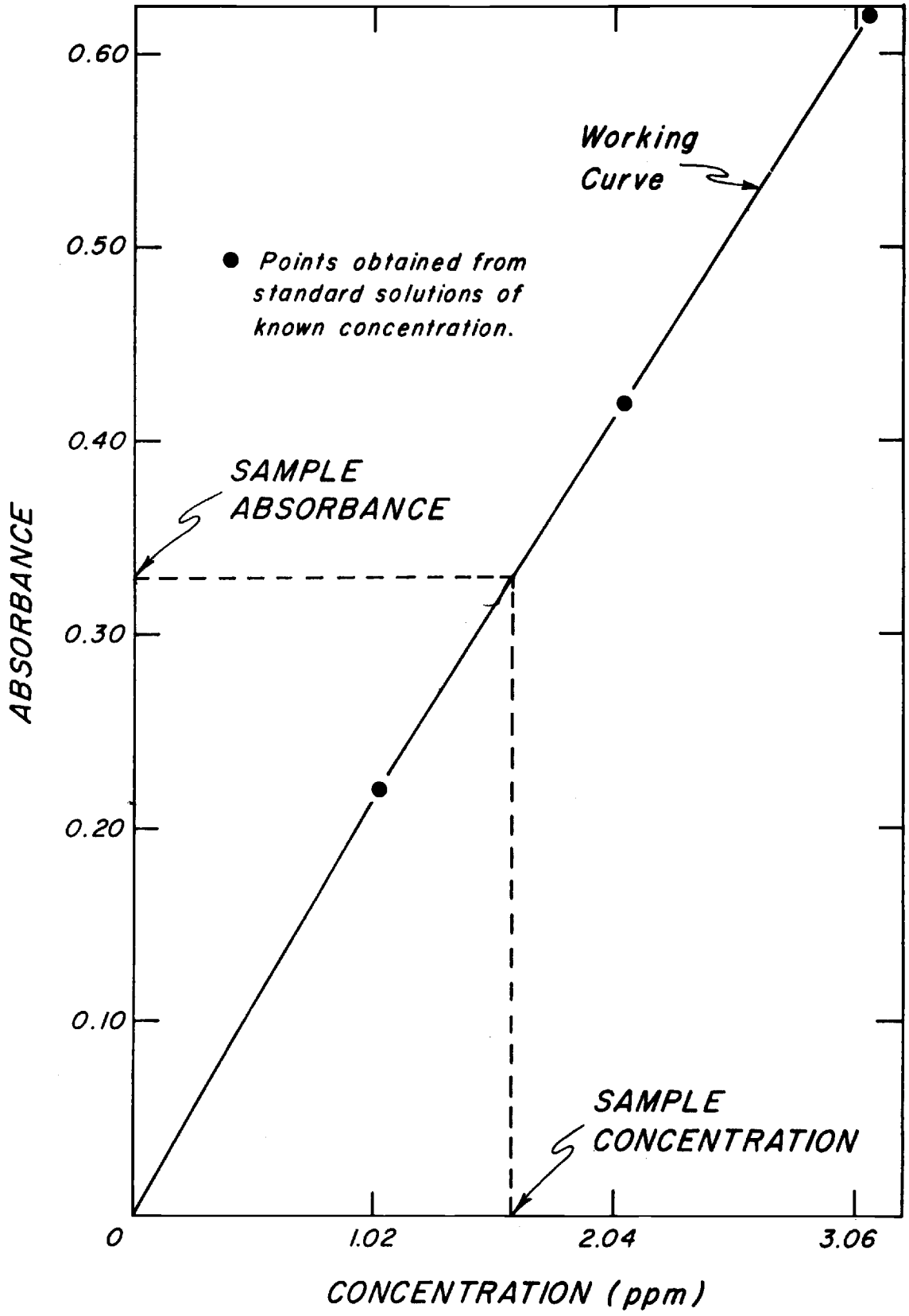

Figure 12. Standard working curve for total zinc determinations

1. Sample weight = 0.05 gm (ash)
2. Sample volume = 25.0 ml
3. Sample concentration (from standard curve) = 1.60 ppm (parts per million i.e. micrograms element/ml sample solution)

The proper computations are:

1. $\frac{1.60 \text{ micrograms Zn}}{\text{ml solution}} \times 25.0 \text{ ml solution} = 40.0 \text{ micrograms zinc}$
2. 40.0 micrograms Zn/0.05 gm sample (ash) =

$$\underline{800 \text{ micrograms Zn/gm sample (ash)}}$$

APPENDIX III

The quantitative results reported in this thesis are in all cases mean (or single) values represented by data points or bar graph tops. There are many factors which affect the precision and validity of the results reported.

The precision of the means reported is affected by inconsistancies introduced by: (1) preparing the samples for analysis e. g. drying, ashing, weighing, and digesting introduce variation from sample to sample; (2) analyzing the samples which gives rise to instrumental variation; and (3) biological variation e. g. one crab gill may contain twice the concentration of a particular element as a different crab tissue collected at the same time. It was found that biological variation influenced the lack of precision moreso than the other two sources of variation. In fact, the first two sources of variation became relatively insignificant. Therefore, a measure of the precision with which the mean values are estimated is reported here as the standard error of the mean ($s_{\bar{x}}$). The standard error of the mean is computed from the radioactivity and total element concentrations of the tissues combined to obtain a datum point or bar graph. The computation is according to:

$$s_{\bar{x}}(\%) = \frac{\sqrt{\frac{\Sigma(x_i - \bar{x})^2}{n(n-1)}}}{\bar{x}} \quad (100)$$

For the sake of clarity, the statistics are computed to correspond with each result figure in the text.

Grand mean (in percent) of the standard error of the mean for bar graphs in Figures 3 and 4. Dashes indicate either single observations or undetectable radioactivity.

Tissue Group	^{65}Zn Radioactivity	^{65}Zn Specific Activity	^{54}Mn Radioactivity	^{54}Mn Specific Activity	^{51}Cr Radioactivity	^{51}Cr Specific Activity
Exterior Tissues	±14	±13	±24	±28	±25	±50
Interior Tissues	±23	±17	±75	±67	±38	±50
Setae	--	--	--	--	--	--

Grand mean (in percent) of the standard error of the mean for bar graphs in Figure 5.

Tissue Group	^{65}Zn Radioactivity	^{65}Zn Specific Activity	^{54}Mn Radioactivity	^{54}Mn Specific Activity	^{51}Cr Radioactivity	^{51}Cr Specific Activity
Metabolically active	±45	±34	±100	±100	±28	±25
Metabolically inert	±31	±40	±44	±42	±24	±13

Standard error of the mean (in percent) for data points in Figure 6. Dashes indicate undetectable radioactivity or single observations.

Tissue Group	^{65}Zn Radioactivity	^{65}Zn Specific Activity	^{54}Mn Radioactivity	^{54}Mn Specific Activity	^{51}Cr Radioactivity	^{51}Cr Specific Activity
Exoskeleton	±14	±17	±15	±11	±15	±36
Mouth Parts	±10	±13	±19	±22	±12	±22
Endophragmal Skeleton	±40	±21	±42	±33	±22	±13
Gills	±17	± 8	±39	±51	±38	±100
Stomach	±13	±18	±90	±88	±44	±37
Soft Shell	±20	±19	±99	±100	±32	±26
Hepatopancreas	±22	±17	--	--	±28	--
Muscle	±12	±10	--	--	±71	±100

Grand mean (in percent) of the standard error of the mean for data points in Figures 7-9. Dashes indicate either single observations or undetectable radioactivity.

Tissue Group	^{65}Zn Radioactivity	^{65}Zn Specific Activity	^{54}Mn Radioactivity	^{54}Mn Specific Activity	^{51}Cr Radioactivity	^{51}Cr Specific Activity
Muscle	±11	±18	--	--	--	--
Hepatopancreas	±30	±37	--	--	±40	--
Gills	±19	±25	±100	±100	±67	±62
Stomach	±34	±48	±100	±80	±81	±85
Exoskeleton	±30	±33	±27	±34	±14	±43
Mouth Parts	±28	±31	±34	±44	± 5	±23